

CLAIMS

1. A method of screening for a substance which modulates  
5 binding of an FHA domain to a phosphopeptide, including:  
    (a) bringing an FHA domain into contact with a  
        phosphopeptide in the presence of one or more test  
        substances;  
    (b) determining binding of the FHA domain to the  
10 phosphopeptide.
2. A screening or assay method for identifying an FHA  
domain which binds to a phosphopeptide of interest, or for  
determining the binding of an FHA domain to a phosphopeptide  
15 of interest including;  
    (a) bringing a test FHA domain into contact with said  
        phosphopeptide; and  
    (b) determining binding of the test FHA domain to the  
        phosphopeptide.  
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3. An FHA domain identified by the method of claim 2,  
which binds to a phosphorylated polypeptide comprising the  
amino acid sequence -Thr(P)-X<sub>1</sub>-X<sub>2</sub>-Asp-, wherein Thr(P) denotes  
a phosphorylated threonine residue, and X<sub>1</sub> and X<sub>2</sub> each  
25 represent any amino acid residue.
4. An FHA domain according to claim 3 comprising an amino  
acid sequence which shares at least 50% homology with the

amino acid sequence of the FHA1 domain of *S. cerevisiae* Rad53p.

5. A screening or assay method for identifying a phosphopeptide which binds to an FHA domain, or for determining the binding of a phosphopeptide to an FHA domain including:

(a) bringing a test phosphopeptide into contact with an FHA domain; and

(b) determining binding of the test phosphopeptide to the FHA domain.

6. A phosphopeptide identified by a method according to claim 5 which binds to an FHA domain.

7. A phosphopeptide according to claim 6 comprising the amino acid sequence -Thr(P)-X<sub>1</sub>-X<sub>2</sub>-Asp- wherein Thr(P) denotes a phosphorylated threonine residue, and X<sub>1</sub> and X<sub>2</sub> each represent any amino acid residue.

8. A phosphopeptide according to claim 6 or claim 7 which binds to the FHA1 domain of Rad53p and/or to Chk2.

9. A phosphopeptide according to any one of claims 6 to 8 comprising the amino acid sequence of a peptide shown in Figure 2.

10. An isolated nucleic acid molecule encoding an FHA domain

according to claim 3 or claim 4 or a phosphopeptide according to any one of claims 6 to 9.

11. A vector comprising a nucleic acid sequence according to  
5 claim 10 operably linked to one or more control sequences.

12. A host cell comprising a vector according to claim 11.

13. A transgenic animal comprising a host cell according to  
10 claim 12.

14. A method according to any one of claim 1, claim 2 or  
claim 5 wherein one or more of the phosphopeptide, FHA domain  
and test substance is in a test sample.

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15. A method according to claim 14 including quantifying the  
amount of phosphopeptide, FHA domain or test substance in  
the sample.

20 16. A method according to any one of claim 1, claim 2 or  
claim 5 including purifying and/or isolating a test substance  
and/or substance of interest from a mixture or extract.

25 17. A method according to any one of claim 1, claim 2 or  
claim 5 comprising labelling one of said FHA domain and  
said phosphopeptide with a detectable label, immobilising  
the other of the FHA domain and the phosphopeptide on a  
solid support and bringing the the FHA domain and the

phosphopeptide into contact.

18. A method according to any one of claims 1, 2, 5 and 14 to 17 wherein the end-point of the assay is phosphorylation  
5 of Rad53p protein.

19. A method of producing an phosphopeptide according to any one of claims 6 to 9 comprising expressing nucleic acid encoding the unphosphorylated peptide and phosphorylating  
10 the expression product.

20. A substance identified by the method of claim 1 which modulates the binding of an FHA domain to a target phosphopeptide.  
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21. A substance according to claim 20 comprising an antibody, single chain antibody or fragment thereof directed to the site of binding in either the FHA domain or the phosphopeptide.  
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22. A substance according to claim 21 wherein said antibody, single chain antibody or fragment thereof is directed at the FHA domain at positions corresponding to Arg-70 and His-88 of Rad53p.  
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23. A substance according to claim 21 wherein said antibody, single chain antibody or fragment thereof is directed at the motif -Thr(P)-X<sub>1</sub>-X<sub>2</sub>-Asp-, wherein Thr(P) denotes a

phosphorylated threonine residue, and  $X_1$  and  $X_2$  each represent any amino acid residue.

24. The use of an FHA domain in screening or searching for,  
5 and/or obtaining or identifying, a phosphopeptide which binds to said FHA domain.

25. The use of a phosphopeptide according to any one of  
claims 6 to 9 in screening or searching for, and/or obtaining  
10 or identifying, an FHA domain which binds to said phosphopeptide.

26. A method of purifying a protein or polypeptide  
comprising an FHA domain able to bind a phosphopeptide, the  
15 method including contacting material containing the polypeptide with a phosphopeptide.

27. A method of purifying a phosphopeptide, the method  
including contacting material containing the phosphopeptide  
20 with a protein or polypeptide comprising an FHA domain.

28. Use of a phosphopeptide which binds to an FHA domain in  
a method of designing a peptidyl or non-peptidyl mimetic of  
the phosphopeptide, which mimetic binds to an FHA domain  
25 and/or modulates interaction between an FHA domain and the phosphopeptide.

29. Use according to claim 28 wherein the phosphopeptide

comprises the amino acid sequence of a peptide shown in Figure 2.

30. Use of an FHA domain in a method of designing a peptide  
5 or non-peptidyl mimetic of an FHA1-like domain, which  
mimetic binds to a phosphopeptide.

31. A method of designing a mimetic of a phosphopeptide  
which has the biological activity of binding to an FHA  
10 domain, or a method of designing a mimetic of an FHA domain  
which has biological activity of binding to a target  
phosphopeptide, said method comprising:

(a) analysing a substance having the biological activity  
to determine the amino acid residues essential and  
15 important for the activity to define a pharmacophore;  
and,  
(b) modelling the pharmacophore to design and/or screen  
candidate mimetics having the biological activity.

20 32. A mimetic obtained by a method of claim 31.

33. The use of a phosphopeptide according to any one of  
claims 6 to 9, an FHA domain or fragment thereof according to  
claim 3 or claim 4 or a substance according to any one of  
25 claims 21 to 23, in the manufacture of a medicament for the  
treatment of a condition associated with a defect or disorder  
in transcriptional control, DNA replication, DNA repair, cell

cycle control or other cellular process mediated by the binding of an FHA domain to a phosphopeptide.

34. The use of a phosphopeptide according to any one of  
5 claims 6 to 9, an FHA domain or fragment thereof according to claim 3 or claim 4 or a substance according to any one of claims 21 to 23, in the manufacture of a medicament for anti-pathogen treatment.

10 35. A pharmaceutical composition comprising one or more of; an FHA domain according to claim 3 or claim 4, a phosphopeptide according to any one of claims 6 to 9, and a substance according to any one of claims 21 to 23.

15 36. A method of treatment of a medical condition associated with a defect or disorder in transcriptional control, DNA replication, DNA repair, cell cycle control or other cellular process, said method comprising; administering a composition according to claim 35.

20 37. A method of treatment of a pathogen infection in an individual, the method comprising; administering a composition according to claim 35 to the individual.